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## The epidemiology of abdominal adiposity

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# **Chapter 7**

## **General Discussion**

## INTRODUCTION

The overall aims of this thesis were to determine the validity of ultrasound (US) measurements of abdominal fat depths compared to Magnetic Resonance Imaging (MRI) or Computer Tomography (CT) measures of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SCAT) in populations covering a wide range of ages; and secondly to apply these measures in large epidemiological studies in order to identify possible early life factors that may influence the quantity of these fat compartments.

In this chapter, the main findings of the studies are first summarised. Methodological considerations are then discussed, followed by the relevance of these findings and clinical and public health implications. Finally, the thesis concludes with directions for future research.

### 1. Summary of the main findings:

#### 1.1 *Validation studies:*

Central adiposity and in particular visceral adipose tissue may play a significant role in the development of obesity related co-morbidities. Direct imaging techniques such as MRI and CT are considered the gold standard methods for the assessment of central adiposity. However, their use is limited in large scale epidemiological studies and in research in young children due to ethical and practical issues. Large-scale epidemiological studies generally implement standard anthropometry and DEXA to assess this adiposity. However, these techniques do not distinguish between different abdominal fat compartments; and anthropometry may be unable to predict racial differences in the distribution of abdominal fat. The results of this thesis suggest that ultrasonography is a suitable non-invasive and reliable tool for quantifying abdominal fat compartments from infancy and in different ethnic groups when compared to direct imaging techniques.

In a sample of 74 older individuals, participating in the Hertfordshire Birth Cohort Physical Activity trial, the ultrasound measures were positively correlated with MRI measures of visceral and subcutaneous abdominal fat (Chapter 2). In this study, the addition of US or ultrasonography improved the prediction of visceral fat over and above the contribution of anthropometry and significantly enhanced the ability to determine the relative quantities of visceral and subcutaneous abdominal fat. However, the use of the prediction models derived in this work may

be limited to populations similar to the one studied here. We therefore determined the accuracy of those models for estimating visceral and subcutaneous abdominal fat in black South African adolescents using MRI as the criterion method (Chapter 3). In this study, ultrasound visceral fat thickness showed the strongest correlations with MRI visceral adipose tissue and significantly increased the estimation of visceral adipose tissue compared to standard anthropometry and DEXA alone. However, the predictions models established in older Caucasian individuals could not be extrapolated to black South African adolescents as this group had relatively little visceral fat thickness and greater subcutaneous fat compared to the elderly Caucasians. In Chapter 4, central adiposity was estimated using anthropometry, DEXA and ultrasound and these estimates were compared against measures of visceral and subcutaneous fat tissues determined by a single slice CT in 6 to 7 years old children. Subcutaneous abdominal fat thickness by US was highly correlated with subcutaneous adipose tissue measured by CT. Surprisingly, there was only weak correlation between US visceral fat thickness and CT visceral adipose tissue. Skinfold measurements showed the strongest correlations with CT visceral and subcutaneous fat measures. DEXA abdominal area was only moderately correlated to visceral fat thickness.

We further validated the ultrasound method in 22 newborn infants and showed that US is a reliable method and can effectively rank infant abdominal fat distribution when compared to MRI measures. Ultrasound measures of visceral fat thickness and subcutaneous abdominal fat were positively correlated with visceral and subcutaneous abdominal adipose tissues measured by MRI (Chapter 6).

## 1.2 *Early life factors:*

The second part of the thesis includes two studies that used the US technique to identify possible early life factors that might influence abdominal fat depositions in the first year of life and in adulthood. Chapter 5 addressed the relationship between birth weight and adult central adiposity and we found that the effects of lower birth weight on increased adult central fat was due to visceral fat rather than subcutaneous abdominal fat. These birth weight associations were dependent on adjustments for BMI suggesting that it is the postnatal transition from lower birth weight to higher BMI that confers increased central fat propensity and likely associated metabolic disease risk, rather than low birth weight *per se*. However, these results only indicate that visceral fat accumulates sometimes between birth and adult life. Therefore, chapter 6,

focused on possible early life determinants that might affect the deposition of these abdominal fat compartments in the first year of life.

In this work, the ultrasound visceral fat thickness significantly increased by 20% between ages 3-12 months and showed weak evidence of tracking between these ages. US visceral fat thickness at both 3 and 12 months were inversely related to skinfold thickness at birth, particularly after adjustment for current skinfold thickness; while US-subcutaneous thickness at 3 months was positively related to skinfold thickness at birth. In addition, infants who were exclusively breastfed at age 3 months had lower US visceral depths at both 3 and 12 months compared to other infants. These findings suggest that both antenatal and postnatal factors may contribute to the quantity of VAT and SCAT in the first year of life.

## **2. Methodological considerations:**

### *2.1 Validation studies:*

#### *2.1.1 Relative versus absolute validity*

In the validation studies, we were unable to directly assess absolute validity (the validity to assess absolute levels of VAT and SCAT from areas or volumes) between the gold standard techniques (MRI and CT) and ultrasonography as these measurements have different units. The US parameters are one-dimensional (depth in cm) and the MRI and CT measures are two or three dimensional (areas in cm<sup>2</sup> or volumes in cm<sup>3</sup>). The Bland and Altman analysis is considered the most appropriate statistical method to test ‘true’ agreement between two instruments as it can reveal both systematic and random errors (1). However, its application is problematic when comparing methods that do not provide results in the same units as only the bias on the raw scale is meaningful. We therefore assessed relative validity by implementing correlation coefficients and predicted absolute values of VAT and SCAT areas or volumes using regression models. Statistical methods such as correlations are conceptually useful as they can tell how consistently a method distinguishes between individuals in a particular population (1) and in this work they are sufficient for ranking individuals according to their abdominal fatness. In the studies described in this thesis, the correlation coefficients between visceral fat thickness and VAT measured by reference imaging techniques ranged between 0.18 to 0.80, and the correlations

between subcutaneous abdominal fat thickness and SCAT ranged from 0.61 to 0.82. In large population based and other epidemiological studies we often tolerate the lack of perfect correlation between the measures of interest and the gold standard techniques due to feasibility and sufficient power due to large sample sizes. Epidemiologic tools such as physical activity and food frequency questionnaires (PAQs and FFQs respectively) have often been found moderately correlated to reference techniques (eg. doubly labelled water and nutrients biomarkers) (2, 3). Findings from other studies have reported correlations between 0.20 to 0.67 with PAQs and total energy expenditure (TEE), and correlations in the region of 0.5 with FFQs and nutritional biomarkers (4-6). However, these methods are extensively used in epidemiology as they suitably rank populations according to their physical activity levels (7) and according to the distribution of intake for specific food groups in etiologic study settings (5). However, these types of questionnaires are poor at determining absolute TEE and absolute intakes of specific nutrients (2, 3, 5, 8).

Overall, due to lack of evidence on absolute validity between ultrasound estimates of abdominal fat and VAT and SCAT measures derived by CT and MRI, and in some cases where better accuracy is required, for instance detailed assessments of individuals with specific diseases or conditions (e.g. lipodystrophy), the use of the gold standard MRI or CT is still valuable.

Future independent studies should then test absolute validity of the prediction equations established in these validation studies using Bland and Altman analysis.

### 2.1.2 *Sample size*

A further limitation is the small sample size of children ( $n=28$ ) (Chapter 4) and infants ( $n = 22$ ) (Chapter 6) in some of these validation studies. Small sample sizes reduce statistical power and the results may be more influenced by one or two outlying values. However, in these studies, the exploration of scatter plots did exclude the presence of such extreme values.

### 2.1.3 *Gold Standards*

In Chapter 6, the validation study in newborns, 3-dimensional volumes calculated from multi-slice MRI images were used to measure VAT and SCAT as the gold standard, or criterion measure of abdominal adiposity. However, because of practical and ethical constraints, the other validation studies were restricted to use a single slice CT or MRI.

This method is less accurate than the whole abdomen imaging method as the inter-individual variation in the distribution of VAT and SCAT across the abdomen is not captured by a single slice (9, 10). VAT and SCAT were determined at the intervertebral space between L4 and L5 (Chapter 4) or at L4 (Chapters 2 and 3). These positions were selected as they corresponded to the location of where the ultrasound depths were measured. However, the vertebral level L2-L3 might be a more suitable position for the quantification of VAT. Several studies have reported that a single slice taken at L2-L3 can more accurately quantify total VAT volumes in adults (11, 12), in different ethnic groups (13) and in children (14) when compared to other single slice estimates (e.g. L4-L5, S1-S2). To capture inter-individual variability of this adiposity, future validation studies, particularly studies containing larger sample of 6-7 years old, should implement MRI volume estimates of VAT and SCAT. If single slice CT is used as the reference method in children studies, investigators should consider determining VAT and SCAT at the vertebral level L2-L3.

## 2.2 *Early life factors studies:*

In the literature several factors have been proposed as possible confounders in the relationship between early growth parameters and body fat distribution: current size, hormonal factors, lifestyle factors, socio-economic status at birth and genetic factors.

### 2.2.1 *Adjusting for current size*

The appropriateness and consequences of adjusting for current size (e.g. weight, BMI) is subject for discussion.

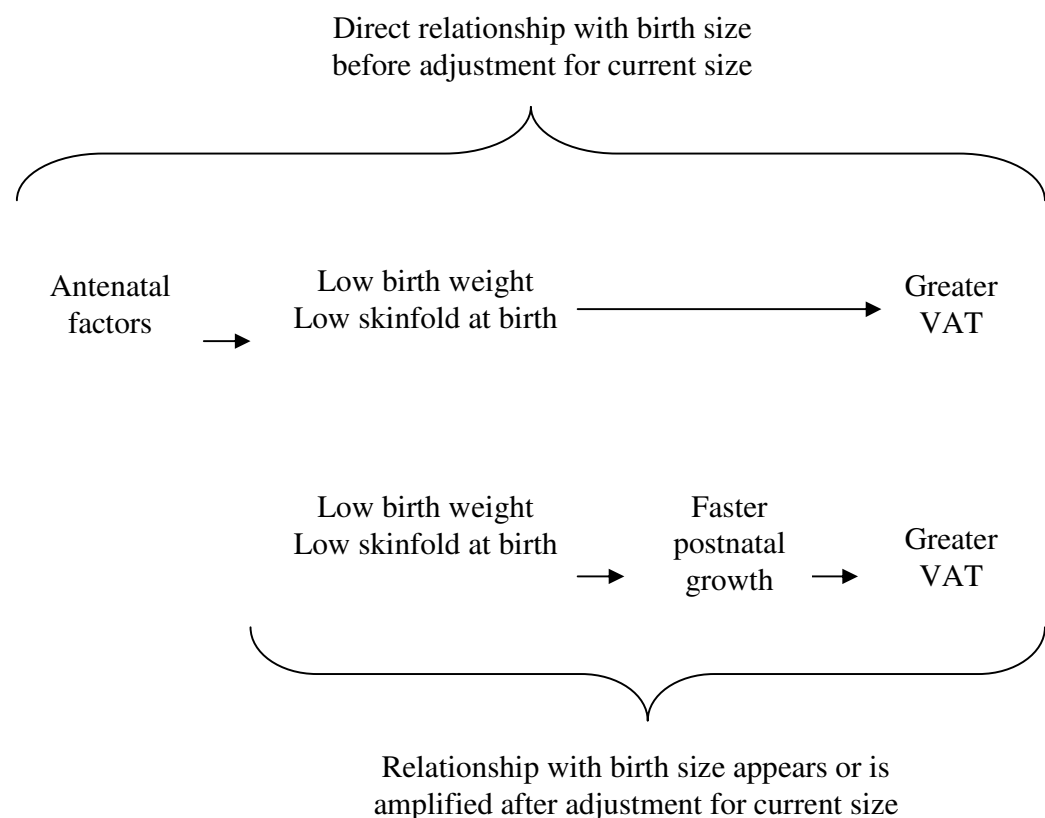
#### 1) Interpretation 1

Tu and colleagues have argued that adjustment for subsequent size could be a form of over-adjustment by creating an artifactual statistical effect known as the “reverse paradox”(15). If current size is in the causal pathway between size at birth and health in later life, adjusting for it may be over-controlling.

#### 2) Interpretation 2

However, most epidemiological studies have used and justified this statistical approach on the grounds that subsequent size is a potential confounder in the relationship in question and that birth size is both related to current size and the

outcome of interest (e.g. central adiposity) (15). However, a transparent approach is required to disentangle the effect of early and later sizes and to identify which one is more relevant to the outcome of interest. Lucas and colleagues have argued that if a relationship with birth weight only exists after adjustment for current weight, then it is the change in weight between birth and follow up that it is implicated rather than fetal biology *per se* (15). Even when birth size is directly related to later health outcome, they caution that studies should explore whether this is partly or wholly explained by postnatal rather than antenatal factors (15). If adjustment amplifies the effect, it is also important to consider whether the change in body size from birth to follow up may be relevant (15). In their report, Lucas and colleagues suggest that all investigations seeking to understand early life programming effects should present both the unadjusted and adjusted models (15).



**Figure 1.** Schematic representation of Lucas and colleagues' interpretation of associations with birth size



Therefore, in this thesis, both unadjusted and adjusted models were presented, and it appeared that the adjustment for subsequent body size was important in the relationships studied: In Chapter 5 the relationship between birth weight and visceral fat thickness in adults became significant only after adjustment for adult BMI; in Chapter 6 the relationships between lower skinfolds at birth and larger visceral fat thickness at 3 and 12 months were present in unadjusted models and were amplified when adjusting for current skinfold thickness, indicating that faster postnatal growth lies in the causal pathway (see Figure 1). In contrast, adjustment for current body size removed the positive relationship between skinfolds at birth and abdominal subcutaneous fat thickness, suggesting that this is due to antenatal factors.

### 2.2.2 *Other potential confounders*

#### *Lifestyle factors*

Postnatal factors such as nutrition might explain or interact with fetal programming effects. The “thrifty phenotype” hypothesis postulates that in the presence of poor maternal nutrition, the foetus adapts to this condition by selecting an appropriate growth trajectory in response to these environmental signals (16). However, if food consumption substantially increases during infancy and childhood resulting in later obesity, the adaptations made by the foetus in uterus become inappropriate for their programming (15, 17). Therefore, it may mean that individuals who are born small in early life, but whose food intake is subsequently increased and their growth consequently accelerated, are more at risk of central adiposity than those who remain small.

Stafford and Lucas have also proposed “the feeding up of the smaller baby” theory (18). They hypothesized that parents of lower birth weight children may choose to feed them more energy dense diets to compensate for their small size at birth (18). Nutrition may therefore mediate associations between early growth and abdominal fat parameters. In the Fenland study, no data were available on infant feeding, therefore it was not possible to determine whether individuals with low birth weight had different mode of feeding than individuals with normal birth weight. Additional analysis in the Cambridge Baby Growth study showed that when adjustment for infant nutrition was included to the model analysing the relationships between early growth measures and abdominal fat parameters,

the regression coefficient did not change significantly. This indicates that nutrition is not a confounder or an explaining variable in these associations. However, our findings also indicate that exclusively breastfed infants had lower visceral fat at 3 and 12 months than mixed fed babies, suggesting that early nutrition may influence the quantity of these abdominal fat compartments in the first year of life.

#### *Socio-economic status*

Socio-economic factors may confound the relationship between early growth parameters and abdominal adiposity as low socio-economic status (SES) has been found to be associated to low birth weight (19-21). In the Fenland study, no information was available on SES at birth. However, adjustment for current education level and smoking (both markers of SES) were taken into account and were added to the final model as these factors were related to current body composition. In the Cambridge Baby Growth study the variable SES was also not available at the point of our analysis. We recognise that this is a limitation.

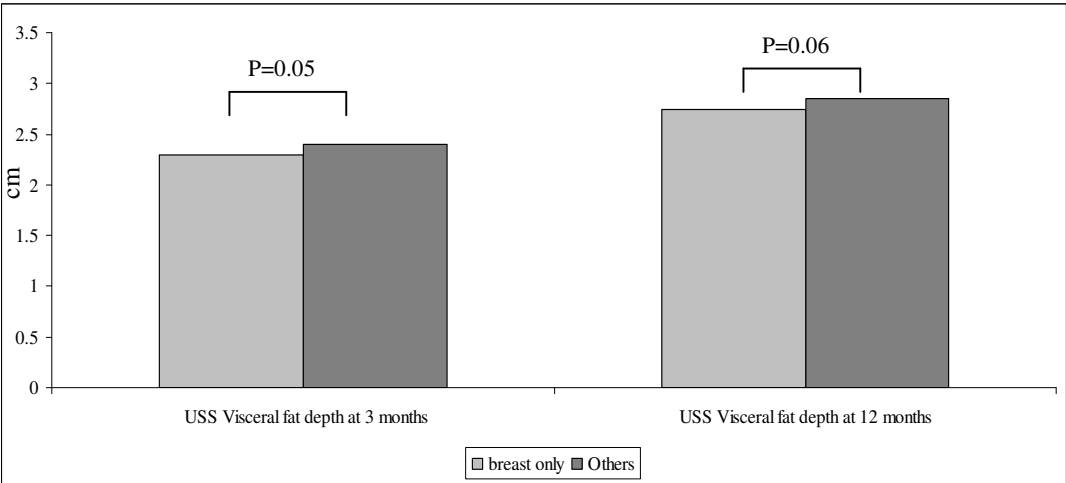
#### 2.2.3 *Multicollinearity:*

Multicollinearity might be a problem in multiple regression analysis when independent variables are highly correlated. It is then difficult to separate the effects of these variables. Body composition variables have been found to be highly correlated in our studies. The diagnostic check, the variance inflation factor (VIF), was therefore used to detect collinearity when constructing prediction models. If VIF was  $>5$  for any two coavariates, only one of them was included in the final model. In Chapter 5, the model containing BMI and total body fat measured by DEXA was invalidated and therefore removed due to collinearity between these two variables.

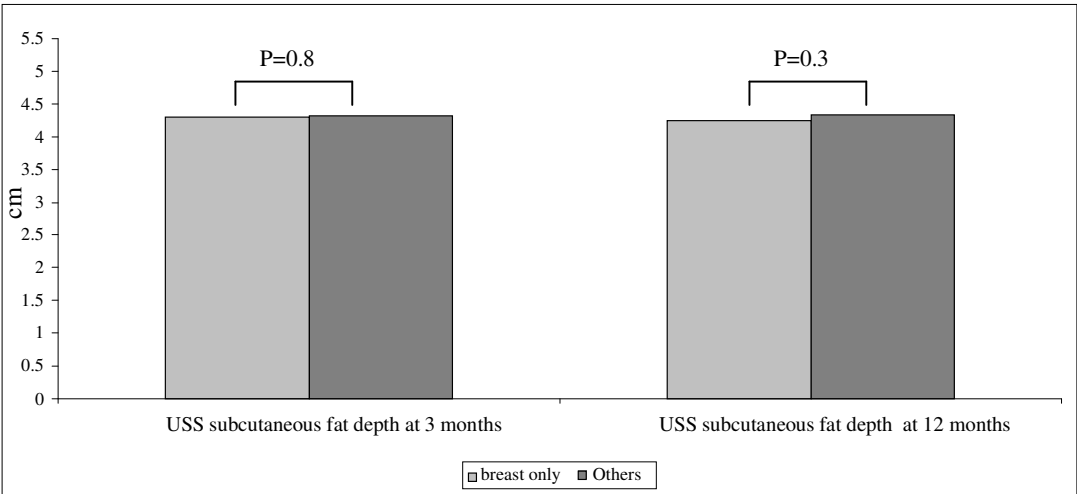
### **3. Mechanisms/regulations of body fat distribution**

The findings of this thesis suggest that, during both intrauterine and postnatal periods, the accumulation of the two abdominal fat compartments VAT and SCAT may be under different regulatory control. Ultrasound visceral fat thickness significantly increased by 20% between ages

3-12 months (Figures 1). In contrast, subcutaneous-abdominal fat remained constant from 3 to 12 months (Figure 2). Other studies have demonstrated that visceral fat deposition may not necessarily be accompanied by subcutaneous fat accumulation, indicating that these two abdominal compartments may result from different regulatory mechanisms (22-24). Factors such as hormonal environment (25, 26), genetic background (27, 28) and early nutrition (29) may play a key role in determining abdominal fat distribution.



**Figure 1.** Visceral fat thickness at 3 and 12 months and nutrition



**Figure 2.** Subcutaneous fat thickness at 3 and 12 months and nutrition

### 3.1 *Hormonal factors*

Underlying hormonal factors may influence regional fat deposition (30). For instance, growth hormone deficiency or hypercortisolemia predisposes to abdominal fat accumulation and unfavorable lipid profiles (31-35). In addition, replacement of growth hormone in growth hormone deficient children as well as adults results in reversal of trunk fat accumulation (36) because of the lipolytic effect of this hormone (37). Flanagan and colleagues have suggested that alterations in growth hormone may mediate associations between lower birth weight and subsequent central obesity (38).

Long term changes in the activation of the hypothalamic-pituitary-adrenal axis may also mediate relationships between birth weight and subsequent adiposity (39). In a study of 370 men ages 59 to 79 years, morning cortisol levels were lower by 26.2 nmol/L (95% CI, 7.2-45.1 nmol/L) for every 1-kg increment in birth weight (40). In a cohort of young South African adults, basal adrenocorticotrophic hormone-stimulated and morning cortisol levels were higher in individuals born at weights below the 10<sup>th</sup> percentile for gestational age compared with those born appropriate for gestational age (41).

Sex steroids have also been shown to affect fat distribution (30). Hyperandrogenicity in women and hypoandrogenicity in men has been associated with an unfavorable fat distribution (42). Sex steroids may also play a role in regulating the accumulation of abdominal fat in infancy when a gonadal activation called ‘mini-puberty’ occurs. In a recent study, male infants who were born premature were found to have higher levels of androgen than full term boys during the first six months of life (43). The authors of this report speculate that through perinatal programming, hyperandrogenism in infancy might have long-term health consequences as it could influence the characteristics of body composition and fat distribution (43).

Endocrine factors could be then involved in the mechanisms that regulate the development of the two abdominal fat compartments and therefore they could potentially confound the relationships between early growth parameters and central fat distribution. In our studies, hormone levels were not determined and therefore, we were not able to study their influence. However, to take into account sex-specific differences in adiposity, our analysis was adjusted for sex, even if the associations between size at birth and fat parameters did not differ by sex.

### 3.2 Genetic factors

Genetic factors might also influence the relationships between birth weight and abdominal fat parameters. Animal knockout experiments have demonstrated the importance of IGF-I, IGF-II and their respective receptors in regulating size at birth (44). It has been reported that variable rates of imprinting and expression of the gene *IGF2R* relate to size at birth (45). In addition, higher levels of the soluble form of IGF2R protein and higher levels of IGF2R relative to IGF-II have been associated with smaller size at birth and placental weight, suggesting that IGF2R may have a growth inhibitory function (45). Finally, a link between absence of a wild type promoter allele in the *IGF1* gene and lower birth weight has been reported (46). This polymorphism is also associated to the risk of obesity and type 2 diabetes (47) and therefore it might be responsible for both low birth weight and increased risk of metabolic complications such as increased abdominal fat compartments. This thesis was not set up to investigate genetic determinants of birth weight and abdominal fat compartments. However, the study of their influences is in the remit of the Cambridge Baby Growth study team.

## 4. Relevance and Clinical/Public health implications

### 4.1 Determinants

Even if it was not possible to determine absolute validity of the ultrasound method, ultrasonography is a reliable method to use from infancy and our observations suggest that it could be used to improve our understanding of the determinants of visceral and subcutaneous fat. I demonstrated that precision greatly increases when ultrasound measures are added to standard anthropometry. This method should then be considered in those studies investigating the interplay of genetic and environmental influences on obesity as accurate and precise measures significantly increase the statistical power to identify the effects of different genes, lifestyle factors and their interactions.

As different associations between body fat and health risk exist in different segments of the population, the application of ultrasonography could help demonstrating the determinants and potential different metabolic sequelae of visceral and abdominal subcutaneous fat depots amongst different ethnic groups.

#### 4.2 *Prediction*

US measures of abdominal fat could be implemented in clinical settings to better predict the risk of obesity and related co-morbidities. Stolk and colleagues have shown that the association between visceral fat thickness measured by ultrasound and the metabolic risk factors of cardiovascular disease is more pronounced than the associations between these factors and waist circumference or waist-to-hip ratio (48). In another report this thickness was found to be associated with metabolic risk factors similar to that measured by abdominal CT scanning (49). Using this method, it may be possible to easily determine abdominal fat depots in clinical practice as it produced more reliable results than simple anthropometry and DEXA and in accuracy closer to abdominal CT or MRI while being relatively cheap, easier to use and more likely to be acceptable by the population. Future studies should also investigate the “predictive ability” of these abdominal fat measures to determine an individual’s risk of adverse health outcomes using tests such as sensitivity and specificity, positive predictive values, net reclassification index and area under the receiver operating characteristic (ROC) curve.

#### 4.3 *Treatment and intervention*

Finally, valid and precise techniques could potentially measure or detect small changes in abdominal fat compartments that could be clinically significant. This is relevant to increase the power of clinical trials that assess the effectiveness of an intervention. Ultrasonography should be then considered in lifestyle interventions to modify regional fat depositions as it could aid lifestyle strategies to improve fat distribution.

### 5. **Main conclusions and directions for future research**

Results from this thesis support the growing evidence that ultrasonography is a valid method to use in epidemiological studies to measure abdominal fat compartments, when the gold standard MRI and CT are not feasible. It is a non-invasive, reliable and reproducible method when compared to these gold standard techniques. It can be used from infancy, making this technique a feasible and valuable method for the assessment of possible risk factors associated with obesity in a very early stage. The use of US in large epidemiological studies could potentially help in exploring the relevance of marked ethnic differences in abdominal fat distribution to metabolic

disease risk and developing future preventative and intervention programs specific to a population group.

Due to lack of evidence on absolute validity, ultrasonography should be used with caution when more accurate measures of fat distribution are required for instance in individuals with specific conditions. For these cases, the use of MRI and CT is still valuable.

Future studies should focus on replication and validation of these findings in different age and ethnic groups, particularly in other infant populations, as our results may be limited to populations with similar characteristic to those we have investigated. These studies should also focus on whether ultrasonography can accurately monitor/detect longitudinal changes in these abdominal fat depositions.

Overall, the findings of the population-based studies presented in this thesis, show that, during both foetal and postnatal periods, visceral and subcutaneous fat compartments may be regulated by different mechanisms. To further investigate the early life determinants of abdominal fat compartments, longitudinal studies with more repeated measures are required to identify potential critical periods of when these compartments develop and whether other covariates such as different mode of infant feeding may modify the relationship between early growth parameters and these adiposities.

Future studies should also assess the clinical usefulness of these measures, for example how they relate to metabolic traits and disease and mortality outcomes. This work should also be carried out in different ethnic groups due to the observed large racial differences in the distribution of abdominal fat.

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